Dynamic karyotype evolution and multiple sex chromosomes in wood white butterflies

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Lepidopteran genomes consist of small-sized holokinetic chromosomes with an ancestral chromosome number of n=31, and a prevalent WZ/ZZ sex chromosome system with female heterogamety. Although the holokinetic nature of lepidopteran chromosomes is supposed to facilitate karyotype evolution mainly due to chromosomal fusions and fissions, recent studies revealed a highly conserved synteny of genes between chromosomes of distantly related taxa and evolutionary stability of karyotypes. The high degree of conservation at the chromosomal level across the phylogenetic tree of Lepidoptera contrasts with exceptional diversity found in some taxa. A typical example is the butterfly genus *Leptidea*, which shows karyotype variability not only between but also within species.

In this work we studied karyotypes of three cryptic *Leptidea* species (*L. juvernica*, *L. sinapis*, and *L. reali*) by means of standard and molecular cytogenetic techniques.

Karyotype differences in chromosome number and structure

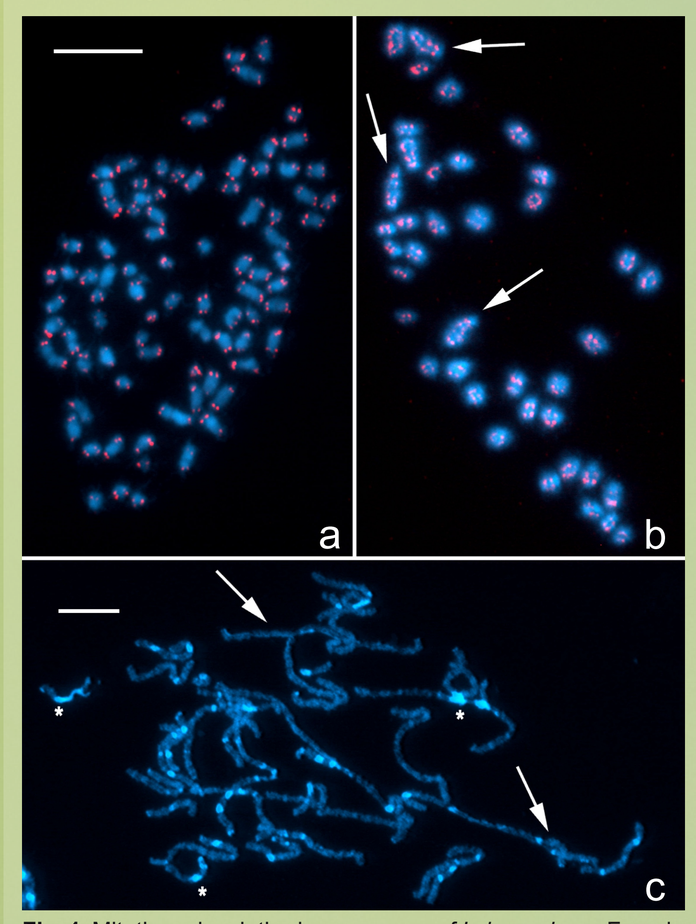

Fig. 1. Mitotic and meiotic chromosomes of *L. juvernica*. **a** Female mitotic metaphase with telomeric signals. **b** Male metaphase I with telomeric signals. **c** Male pachytene complement. Chromosomal multivalents (arrows); heterochromatic blocks (asterisks). Bar = 10 µm.

- Chromosome number ranged from 2n=85-91 in *L. juvernica* (Czech population) and 2n=69-73 in *L. sinapis* (Czech population) to 2n=51-55 in *L. reali* (Spain population).
- Differences in chromosome number were observed even in the progeny of individual females.
- We observed multiple chromosomal fusions and other complex rearrangements in all three species, often accompanied by the occurrence of large heterochromatic blocks.

Sex chromosome constitution

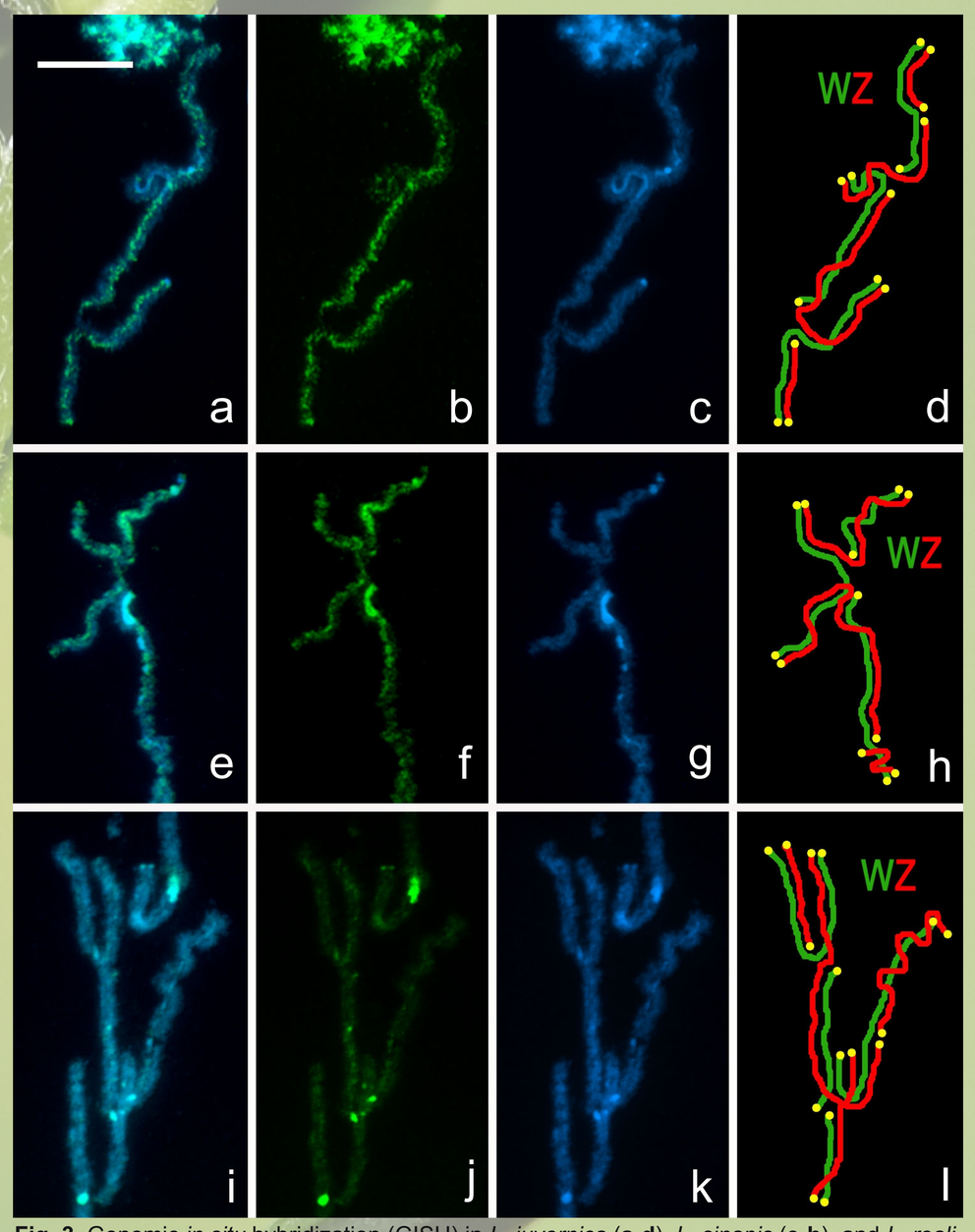

Fig. 3. Genomic *in situ* hybridization (GISH) in *L. juvernica* (**a-d**), *L. sinapis* (**e-h**), and *L. reali* (**i-l**). **a**, **e**, **i** DAPI stained chromosomes merged with female genomic probe. **b**, **f**, **j** Female genomic probe. **c**, **g**, **k** Sex chromosome multivalents stained with DAPI. **d**, **h**, **l** Schematic drawing of sex chromosome constitution with yellow telomeres. Bar = 10 µm.

- GISH revealed multiple sex chromosomes in pachytene oocytes of all three species, with the following constitutions:
  - $W_1W_2W_3Z_1Z_2Z_3Z_4$ in *L. juvernica*
  - $W_1W_2W_3Z_1Z_2Z_3$ in *L. sinapis*
  - $W_1W_2W_3W_4Z_1Z_2Z_3Z_4$ in *L. reali*

Localization of cytogenetic markers

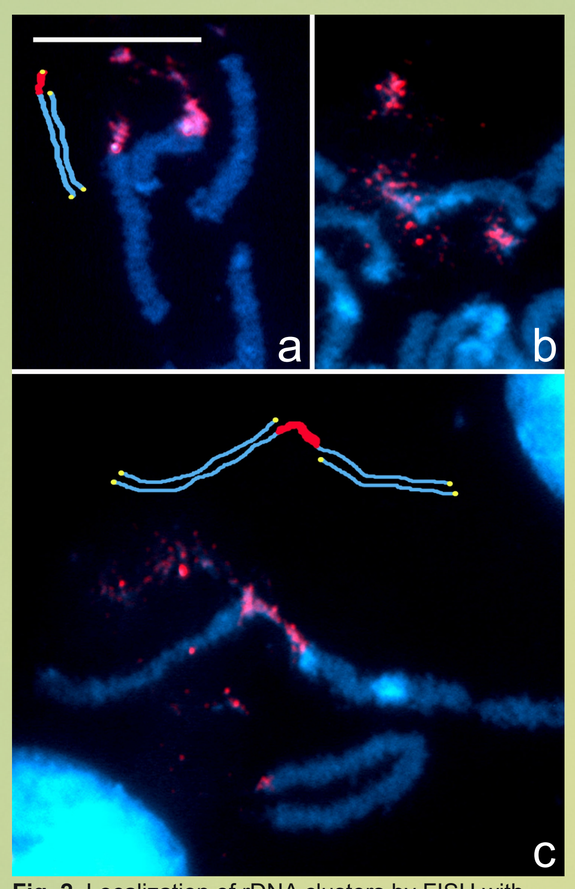

Fig. 2. Localization of rDNA clusters by FISH with 18S rDNA probe (red) in pachytene chromosomes of *L. sinapis* (**a**, **b**) and *L. juvernica* (**c**). Bar = 10 µm.

- We found significant differences in the location of major rDNA clusters among the three studied *Leptidea* species.
- In *L. juvernica* and *L. sinapis*, the location of rDNA differed between the offspring of individual females.
- We also found inter- and intraspecific differences in the number and location of otherwise conserved H3 histone gene clusters.

Conclusions

We confirmed inter- and intraspecific karyotype differences in chromosome number and structure in three closely related wood white butterflies. The karyotype variability is likely due to irregular chromosome segregation of multivalent meiotic configurations. Our results suggest a dynamic karyotype evolution and point to the role of chromosomal rearrangements in speciation of *Leptidea* butterflies. Moreover, our study revealed a curious sex chromosome constitution with 3-4 Z and 3-4 W chromosomes, which is unique not only for butterflies but for entire animal kingdom. These multiple sex chromosomes could play an important role in the formation of reproductive barriers between populations of white butterflies; however, this hypothesis should be verified by further research.

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